328-Pos Board B128
Characterizing Intrinsically Disordered Proteins by Small-Angle Neutron Scattering
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Intrinsically disordered proteins (IDPs) that undergo a coupled folding and binding event are found to be important in many recognition and signaling processes. However, exactly how this mechanism is linked to these functions is not completely understood. This is primarily because the structural flexibility of IDPs limits the number of suitable characterization techniques. We are using small-angle neutron scattering (SANS) to investigate the structure and binding interaction properties between NCBD, an IDP region of CREB binding protein (CBP), and its binding partner, ACTR, which also is an IDP. CBP is a transcription co-activator that is essential in embryonic development, growth control, and homeostasis, and its dysfunction is implicated in neurological disorders such as Huntington's disease and some cancers. SANS indicates the NCBD/ACTR complex is a globular, folded structure with a smaller radius of gyration compared to ACTR alone, which is mostly unfolded. Using *ab initio* shape reconstruction programs to gain further insight into structural flexibility, we find good agreement between the shape reconstruction and NMR structure for the NCBD/ACTR complex. In contrast, SANS reveals the nature of how ACTR is more expanded when in its unbound, unfolded state. This research should provide new possibilities for the study of disordered protein regions and yield unique perspectives into the mechanism of IDP function.

329-Pos Board B129
Mapping Ntail-XD Binding Interactions Between Paramyxoviral Proteins Using Cyanylated Cysteine
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Cyanylated cysteine is a site-specific vibrational probe of structural transitions in intrinsically disordered proteins. The CN stretching band broadens when located near structural formation events, and it also can shift in frequency due to water exclusion by binding partners. The relatively well-characterized interaction between the disordered measles nucleoprotein C-terminus (N_{TAIL}) and the measles phosphoprotein X domain (XD) was used to demonstrate the sensitivity of the probe to local binding at several sites along measles N_{TAIL}. Single cysteine variants of disordered N_{TAIL} proteins from the hendra and nipah viruses have been expressed and cyanylated at various positions to map binding interactions with their respective XD's. This approach to identifying sequence regions involved in binding events will establish the utility of site-directed mutagenesis and cyanylation of cysteine as a screening strategy for determining binding-induced structural transitions in all IDPs.

330-Pos Board B130
Nitration of Tyrosine Residues in Alpha-Synuclein Reduces Vesicle Binding Through Electrostatic and Allosteric Effects
Eva Sevcsik, **David C. DeWitt**, Adam Trexler, Elizabeth Rhoades.
Lewy body plaques found in the brains of Parkinson's disease patients are composed mainly of the protein alpha-synuclein (aS). Oxidative damage has been linked to pathogenesis, and oxidatively modified aS, specifically aS nitrated at tyrosine residues, has been found in fibers and insoluble aggregates extracted from brain tissue. It has also been shown that nitrated aS has weaker binding to lipid membranes than non-nitrated aS. We have studied the effect of oxidative modification on monomeric aS by fluorescence correlation spectroscopy (FCS) and single molecule Förster resonance energy transfer (smFRET). There are four tyrosine residues in aS that can be oxidatively modified through nitration, one in the N-terminal membrane binding region and three in the C-terminus, which does not interact directly with membranes. Selective nitration of the tyrosines in either region results in a similar reduction in lipid binding affinity. The change in affinity upon nitration of the N-terminal tyrosine is the result of the increased electrostatic repulsion. The reduction in binding affinity upon nitration of the C-terminal tryosines appears to be an allosteric effect mediated by a change in the conformation of the C-terminus. This observation may reflect a more general mechanism for modulating interactions between aS and cellular membranes through modification of the C-terminus.

331-Pos Board B131
Alterations to the Conformational Ensemble and Intermolecular Associations of Polyglutamine Due to Charged Side Chains at the N- and C-Termini
Nicholas J. Lyle, Scott L. Crick, Rohit V. Pappu.
Expanded polyglutamine (polyQ) tracts are associated with several neurodegenerative disorders including Huntington's disease (HD). Biophysical and computational characterization of polyQ peptides has been used to probe the intrinsic properties of polyQ and to study the mechanism by which polyQ aggregates. Experiments with the peptide construct $K_2Q_NK_2$ show a pronounced lag phase. This has led to the proposal that polyQ peptides aggregate via a homogeneous nucleation mechanism. Most *in vitro* kinetics experiments with polyQ have been performed with terminal lysine residues to enhance solubility. It has been assumed that these charged residues do not affect the properties of the polyQ peptide or the mechanism of aggregation. Recent simulations and experiments call this assumption into question.
We present results from computational studies on the effects of charged side chains at the N- and C-termini to understand how the intrinsic length dependent properties and intermolecular interactions of polyQ constructs are altered by terminal charges. This study is based on simulations utilizing the ABSINTH solvation model. The results show that below a certain length scale, the conformational ensemble of polyQ is altered by finite size and charge effects and the intrinsic polymeric behavior of polyQ is recovered at high molecular weights. These results make the point that simple polymeric models, such as the worm-like-chain model, fail to capture these trends and their application to polyQ systems is dubious. PolyQ intermolecular interactions are altered by long-range electrostatics and this persists over a large length scale. Lastly, we show that the charged terminal residues alter the amplitudes of intra-polyQ conformational fluctuations. Since these fluctuations are involved in modulating aggregation, this yields insight into how polyQ flanking sequences play a role in the mechanism of polyQ aggregation.

332-Pos Board B132
Intrinsic Helicity of Disordered Basic Regions of Bzip Transcription Factors: Implications For Mechanisms of DNA Binding
Rahul K. Das, Scott L. Crick, Rohit V. Pappu.
Transcription factors (TFs) are enriched in disorder promoting residues. Basic leucine zipper (bZip) TFs comprises of a basic DNA binding region (bR) and a leucine zipper region (LZ). It is believed that bZip monomers are intrinsically disordered; this is especially true of the bRs. Folding of bZips is assumed to be coupled to DNA binding. The mechanism of conformational change coupled to DNA binding remains unresolved. Here, we report results from a systematic computational and experimental studies to scrutinize the role of bRs of the bZip TFs in DNA binding. Our results show that bR sequences encode varying extents of helicity that are confirmed by comparing simulation results to UV-CD measurements. Partial helicity in bRs derives from intrinsic helicity for the consensus 10-residue stretch, which we refer to as DNA binding signature sequences (bRSS). This element or aMoRE is flanked by N-terminal acidic groups and C-terminal basic groups, which act as helix capping boxes and the overall helicity within bR is modulated by the nature of these charged caps. Furthermore, our simulation results revealed that the overall helicity of the bZIP show strong positive correlation with the helicity of the bR and weak positive correlation with the helicity in LZ while the helicities in bR and LZ are also weakly correlated. These findings lead us to propose a phenomenological model for DNA binding of bZip. DNA binding can occur via at least two parallel channels, and the proposal is that flux through the two channels is controlled by both the intrinsic helicity in the BR and the stability of the dimer formed by LZ of bZips.

333-Pos Board B133
Modeling Alpha-Synuclein Ensemble Using Bayesian Weighting Algorithm
Orly Ullman, Collin M. Stultz.
Alpha-Synuclein is an intrinsically disordered protein (IDP) shown to be implicated in the pathogenesis of Parkinson's disease. IDPs do not adopt a well defined secondary structure; instead they are thought to adopt a heterogeneous collection of conformations in solution. A combination of a collection of conformations and their relative stabilities - an ensemble - allows insight into the complex energy landscape of IDPs. In this work we use a Bayesian weighting algorithm developed in our lab to model the ensemble of alpha-Synuclein. We incorporate experimental data from previously published work together with molecular dynamics simulations to form an ensemble that agrees with experiment. By using the Bayesian approach we do not rely on a single set of weights but rather on a distribution over possible sets of weights. In addition the Bayesian approach allows for a novel uncertainty measure, by which we can quantify our confidence in predictions made using the ensemble. Alpha-Synuclein was shown to aggregate in vitro and was found to be the major component in neuronal inclusions known as Lewy bodies. It is believed that the formation of alpha-Synuclein aggregates has a toxic effect on dopaminergic neurons. Residues 68-78 in alpha-Synuclein were shown to be a critical component for alpha-Synuclein aggregation and toxicity. We present preliminary results analyzing long-range contacts within alpha-Synuclein. We find alpha-Synuclein forms multiple long-range contacts throughout the sequence; however, residues 68-78 form comparatively few long-range contacts with distant residues within alpha-Synuclein. In addition we find that this region has a rather large solvent accessible surface area of 60% its maximum value.